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Interaction and Dissolution Characteristics of Ajmaline-PVP Coprecipitate[1]

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The ajmaline-PVP coprecipitates were prepared for the purpose of increasing the solubility of ajmaline. The dissolution rate of 1: 5 w/w coprecipitate was approximately 130-fold enhanced compared with ajmaline.

In nuclear magnetic resonance (NMR) spectrum of ajmaline, the signal of C(21)-proton was shifted to lower magnetic field by polyvinylpyrrolidone (PVP), and 2-pyrrolidone showed the same effect on ajmaline, while NMR spectrum of 17,21-diacetylajmaline was not affected by PVP. On addition of PVP, the λ_{max} of ajmaline shifted to shorter wavelength in chloroform, but ultraviolet (UV) spectra of diacetylajmaline were not affected. By 2-piperidine, the shift of C(21)-proton signal of ajmaline in NMR spectra could not be detected. From these results, C(21)-hydroxyl groups of ajmaline were considered to form weak hydrogen bond to carbonyl groups of pyrrolidone rings of PVP.

The coprecipitate and the physical mixture of PVP and diacetylajmaline, in which the interaction could not be detected as far as spectroscopic investigation was concerned, were also prepared. The dissolution rate of diacetylajmaline was enhanced in the coprecipitate and slightly in the physical mixture compared with that of diacetylajmaline alone.

From the solubility study and the equilibrium dialysis study, it was suggested that one PVP molecule interacts with two or three molecules of ajmaline or diacetylajmaline in aqueous solution.

The dissolution characteristics of ajmaline-PVP coprecipitate were discussed in comparison with diacetylajmaline-PVP coprecipitate.

Keywords——polyvinylpyrrolidone; coprecipitate; ajmaline; 17,21-diacetylajmaline; dissolution rate; UV and NMR spectra; solubility method; equilibrium dialysis study

Enhancing dissolution rates of poorly soluble drugs is of great pharmaceutical importance. Tachibana, *et al.*[3] reported a new method of preparing aqueous dispersion of β-carotene by using water soluble polymer like polyvinylpyrrolidone (PVP). They presumed that β-carotene was molecularly dispersed in solid of PVP, and that PVP acted as a solid solvent for β-carotene. Mayersohn, *et al.*[4] also reported that the dissolution rate of griseofulvin-PVP solid solution resulted in a 5- to 10-fold increase compared to that of griseofulvin alone. Bates, *et al.*[5] ascertained the possibility of enhancing the dissolution and absorption rates of reserpine by reserpine-PVP coprecipitates. However the knowledge on the detailed mechanism of interaction of PVP with water insoluble drug is relatively scarce.

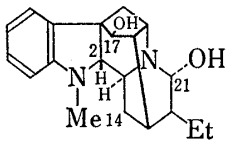

Chart 1. Structure of Ajmaline

In this study, the coprecipitates of ajmaline and PVP were prepared, and the dissolution characteristics were examined. The interaction sites of ajmaline and PVP were studied by ultraviolet (UV) absorption spectra and

1) Presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, Apr. 1975.
2) Location: *1432–1, Horinouchi, Hachioji, Tokyo, 192–03, Japan.*
3) T. Tachibana and A. Nakamura, *Kolloid-Z. Polym.*, **203**, 130 (1965).
4) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **55**, 1323 (1966).
5) T.R. Bates, *J. Pharm. Pharmacol.*, **21**, 710 (1969); E.I. Stupak and T.R. Bates, *J. Pharm. Sci.*, **61**, 400 (1972).

nuclear magnetic resonance (NMR) spectra. In addition, the interactions of ajmaline and PVP in aqueous solution were also investigated by equilibrium dialysis and solubility method.

Experimental

Materials——Polyvinylpyrrolidone (PVP) K-15 was refluxed with ether for 24 hours for the purpose of removing PVP of lower molecular weight. The mean molecular weight was evaluated to be 7200 by viscometry. Ajmaline, 2-pyrrolidone, and 2-piperidine employed in this study were of commercial grades. 17,21-Diacetylajmaline was synthesized from ajmaline with acetic anhydride according to the procedure of Anet, *et al.*[6] N-Methylindoline was also prepared from indoline by standard procedure. All other chemicals were of JIS special grades of the like, and were used with further purification if necessary.

Preparation of Coprecipitates——Ajmaline-PVP coprecipitates, in ratio of 1:2, 1:4, and 1:5 w/w, were prepared by dissolving both of the components in chloroform and the solvent was subsequently evaporated, and then the residues were dried *in vacuo* to constant weight. The particles passed through the sieve of 250 mesh were collected for use in the dissolution rate studies. The 1:5 w/w physical mixture, pure ajmaline, and its chloroformate were also screened through the same sieve and subjected to dissolution rate testing. The coprecipitate and physical mixture of diacetylajmaline and PVP were prepared in the same manner as those of ajmaline and PVP.

Dissolution Rates——A 500 ml three neck round bottom flask containing 300 ml of distilled water was used as the apparatus for dissolution test and maintained at 37±1°. The solution was stirred at 150 rpm. After the quantity of each of the samples equivalent to 500 mg of ajmaline was introduced into the medium, an aliquot (1 ml) of the dissolution medium was taken through a Millipore filter periodically and assayed. Both dissolved ajmaline and diacetylajmaline were assayed with a Hitachi model 124 spectrophotometer at wavelength 288 nm. PVP present in the sample solution did not interfere with the determination of ajmaline and diacetylajmaline.

Physicochemical Determination of Binding Characteristics——The UV absorption spectra of ajmaline and diacetylajmaline in chloroform and aqueous solution were measured with a Hitachi model 323 spectrophotometer. NMR spectra of the samples in deuterochloroform were also measured with Varian model T-60.

The evidence of interaction in aqueous solution was ascertained by solubility method[7] and equilibrium dialysis method.[8] In solubility study an excess of ajmaline was added to 30 ml of PVP aqueous solution of the various concentrations in glass-stoppered bottles, which were maintained at constant temperatures of 0°, 15°, and 30°. The concentration of ajmaline was determined after equilibrium was reached (48 hr). In equilibrium dialysis study, 1% solution of PVP and various concentrations of ajmaline solution, were separated by the Visking film in the container prepared by acrylics. The dialysis was carried out for 48 hours at 0°, 15°, and 30°. The concentrations of ajmaline in the solution devoid of PVP and containing PVP were determined. The dialysis experiment of diacetylajmaline was carried out in the same way at 0° and 30°.

Results

1) Dissolution Rate of Ajmaline

The dissolution characteristics of ajmaline, its chloroformate, ajmaline-PVP physical mixture and coprecipitates, are shown in Fig. 1. The dissolution rate of ajmaline from 1:5 coprecipitate was significantly enhanced, compared with those of 1:5 physical mixture, ajmaline or the chloroformate. The more the contents of PVP increased, the higher were the dissolution rate and the solubility of ajmaline. In the physical mixture too, the dissolution rate of ajmaline tended to increase compared with those of ajmaline alone or its chloroformate. The apparent solubility of 1:5 physical mixture was one-half of that of 1:5 coprecipitate after 60 minutes. The dissolution rate of the chloroformate was much the same as that of ajmaline.

2) Interaction of PVP and Ajmaline in Chloroform Solution

The maximum wavelength (λ_{max}) of UV spectrum of ajmaline were 252 nm and 296 nm in chloroform solution. The λ_{max} of higher wavelength of ajmaline was shifted by 2 nm to shorter wavelength by addition of PVP as illustrated in Fig. 2.

6) F.A.L. Anet, D. Chakravarti, R. Robinson, and E. Schlittler, *J. Chem. Soc.*, **1954**, 1242.
7) T. Higuchi and D.A. Zuck, *J. Am. Pharm. Assoc.*, **42**, 132 (1953).
8) T. Higuchi and R. Kuramoto, *J. Am. Pharm. Assoc.*, **43**, 393 (1954).

On the NMR spectra of ajmaline in deuterochloroform, the signal at 4.2 ppm of δ was shifted to lower magnetic field by addition of PVP. With 2-pyrrolidone, the monomeric analogue of PVP, the signal at 4.2 ppm was shifted similarly and the signal at 2.77 ppm was shifted slightly to higher magnetic field as shown in Fig. 3.

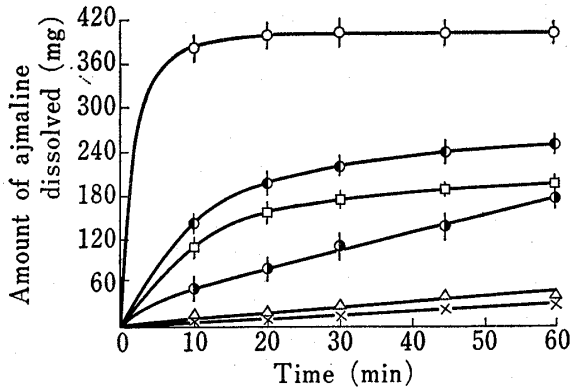

Fig. 1. Dissolution Rate of Ajmaline, Its Chloroformate, and Coprecipitates and Physical Mixture with PVP at 37° in Water

Key: △, ajmaline. ○, 1:5 coprecipitate.
×, ajmaline chloroformate. ◐, 1:4 coprecipitate.
□, 1:5 physical mixture. ◑, 1:2 coprecipitate.
Results are expressed as the mean ± S.D. of four experiments.

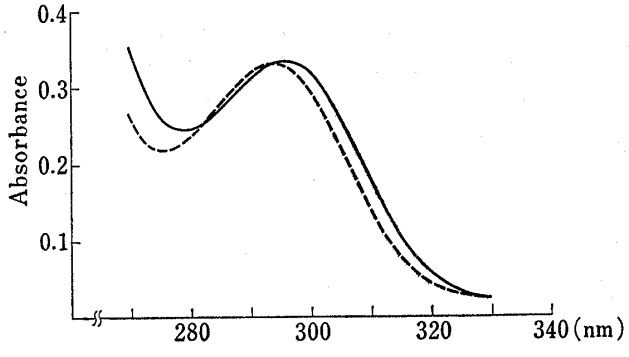

Fig. 2. UV Absorption Spectra of Ajmaline in the Presence and Absence of PVP in Chloroform

Key: ——, ajmaline.
– – –, ajmaline and 1×10^{-3} M PVP.

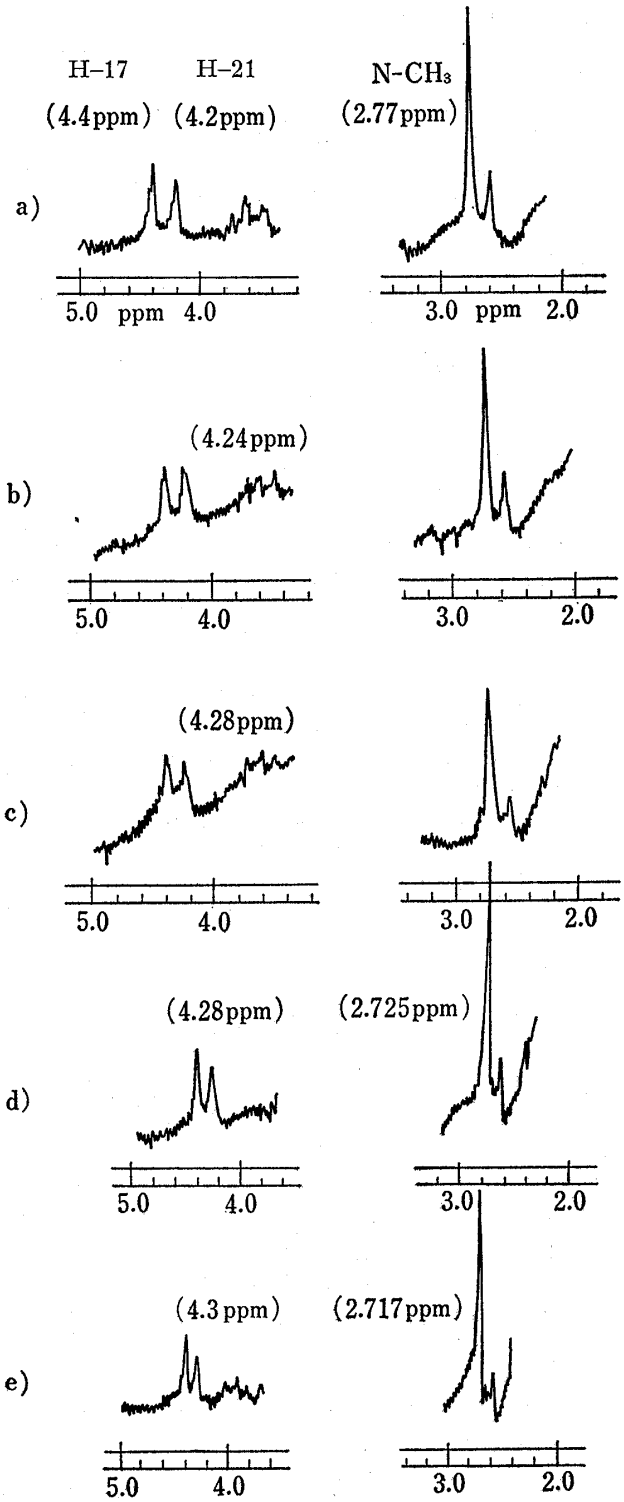

Fig. 3. NMR Spectra of 0.2 M Ajmaline in $CDCl_3$

a) Absent of PVP or 2-pyrrolidone.
b) 0.0025M PVP.
c) 0.005M PVP.
d) 0.25M 2-pyrrolidone.
e) 0.5M 2-pyrrolidone.

On the other hand, the λ_{max} of UV spectra of diacetylajmaline was identical with that of ajmaline in chloroform solution, but the λ_{max} was changed by neither PVP nor 2-pyrrolidone. In the NMR spectra of diacetylajmaline, the effect of PVP or 2-pyrrolidone could not be detected either.

3) Disolution Rate of Diacetylajmaline

The dissolution characteristics of diacetylajmaline, diacetylajmaline-PVP physical mixture and coprecipitate, are shown in Fig. 4. The dissolution rate of diacetylajmaline from coprecipitate was more remarkably enhanced, compared with those of physical mixture and diacetylajmaline alone. The solubility reached the maximum value and then decreased gradually with the elapse of time. After 30 minutes, it approximately reached the equilibrium state. The solubility of 1: 5 coprecipitate after 60 minutes was about twice as high as that of pure diacetylajmaline.

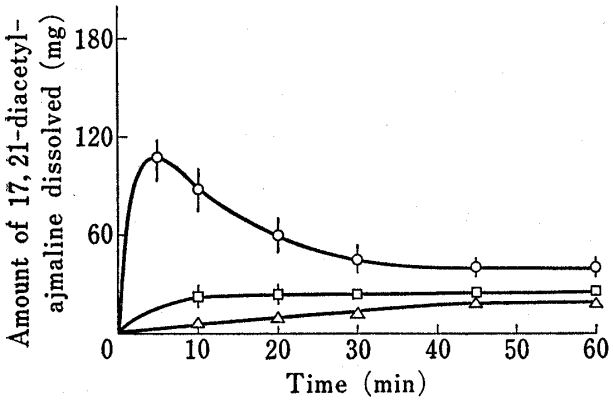

Fig. 4. Dissolution Rate of Diacetylajmaline at 37° in Water

Key: △, diacetylajmaline.
○, 1:5 coprecipitate.
□, 1:5 physical mixture.
Results are expressed as the mean ± S.D. of four experiments.

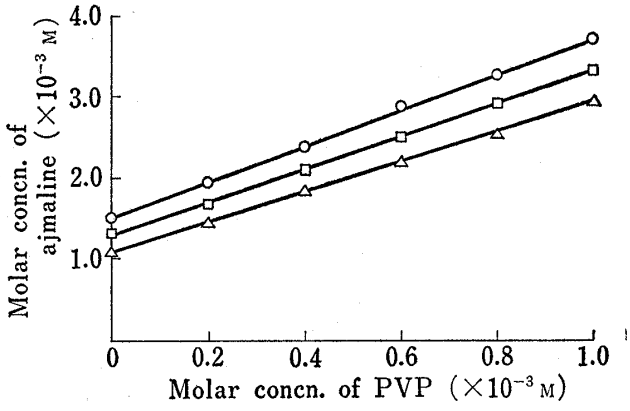

Fig. 5. Aqueous Solubility of Ajmaline in the Presence of PVP

Key: △, 0°; □, 15°; ○, 30°.

4) Interaction of PVP and Ajmaline or Diacetylajmaline in Aqueous Solution

In aqueous solution, the λ_{max} of ajmaline or diacetylamjaline were 244 nm and 288 nm, and the λ_{max} of higher wavelength of ajmaline or diacetylajmaline was not changed by addition of PVP or 2-pyrrolidone.

However, as shown in Fig. 5, the solubility of ajmaline increased linearly with the increase in the concentration of PVP at each temperature. The slopes of these straight lines tended to rise slightly with a rise in temperature. From these slopes, it seems that the average number of binding sites for ajmaline on a PVP molecule is approximately 2.4 in aqueous solution.[9]

From the equilibrium dialysis, three straight lines were obtained by Langmuir plots. They passed through approximately the same intercept at each temperature. From this intercept, the number of binding sites on a polymer molecule was about 2.7 (Table I); *i.e.*, 24 monomer units of PVP provided a binding site for one ajmaline molecule.

Results from the equilibrium dialysis of PVP and diacetylajmaline in aqueous solution showed that the number of binding sites on a polymer molecule was about 2.4, *i.e.*, 27 monomer units of PVP were required for one ajmaline molecule (Table I).

9) M.J. Cho, A.G. Mitchell, and M. Pernarowski, *J. Pharm. Sci.*, **60**, 720 (1971).

Discussion

PVP is the water soluble polymer and is known to be pharmacologically inert and to interact with various drugs. In view of these properties of PVP, it seems that these approaches to the clarification of interaction mechanisms between PVP and ajmaline may be important and may lead to some significant biopharmaceutical application.

From this experiment, the initial dissolution rate constants of 1: 5 coprecipitate, 1: 5 physical mixture, and ajmaline alone, were observed to be 2.99 min^{-1}, 0.08 min^{-1}, and 0.023 min^{-1} respectively. These results indicated an approximately 130-fold increase in the dissolution rate of ajmaline from the 1: 5 coprecipitate, and 3.5-fold increase from the 1: 5 physical mixture over that for the pure ajmaline. These enhanced dissolution characteristics of ajmaline from 1: 5 coprecipitate did not seem to be due to the crystallinity change of ajmaline molecules by chloroform or merely the solubilizing action of PVP. Consequently, it may be considered that PVP exerts some effect on ajmaline.

Concerning the interaction sites of PVP and ajmaline, it would be expected that the terminal vinyl groups or the lactam groups of pyrrolidone rings in PVP influence the hydroxyl groups at the C(17)- and C(21)-positions or nitrogen atoms at the N(1)-position in ajmaline. To clarify this point, the UV and NMR spectra of ajmaline were investigated. By addition of PVP, the λ_{max} of ajmaline was shifted to shorter wavelength by 2 nm in chloroform (Fig. 2). In NMR spectrum study of ajmaline, Muquet, *et al.*[10] reported that the signal of C(17)-proton of ajmaline was found in lower magnetic field than that of C(21)-proton, because C (17)-proton influenced axial C(2)- and C(14)-protons owing to cage structure of ajmaline. Therefore the signals of 4.2 and 4.4 ppm obtained from this experiment were attributed to C(21)- and C(17)-proton respectively. As shown in Fig. 3, the signal of C(21)-proton was shifted to lower magnetic field by 0.08 ppm, but the signal of N(1)-methyl group corresponding to 2.77 ppm was not shifted by addition of PVP. Furthermore, with 2-pyrrolidone, the signal of C(21)-proton was shifted to lower magnetic field by 0.1 ppm and the signal of N(1)-methyl group was shifted to higher magnetic field by 0.053 ppm (Fig. 3). From these results, it became apparent that 2-pyrrolidone exerts on ajmaline the similar effect to PVP. On the other hand, the UV and NMR spectra of diacetylajmaline, in which C(17)- and C(21)-hydroxyl groups of ajmaline were acetylated, were not affected by addition of PVP or 2-pyrrolidone.

N-Methylindoline was subjected to UV absorption studies as the chromophore of ajmaline, though it differs from ajmaline in the lack of hydroxyl groups and of strain in conformation. It would be considered that the N-methylindoline serves as substitutes of ajmaline for studies of the interaction of PVP and ajmaline. Both UV and NMR spectra in chloroform were not at all influenced by either PVP or 2-pyrrolidone. The influence of 2-piperidine, which has no carbonyl groups unlike 2-pyrrolidone, was investigated on the NMR spectra of ajmaline, but the shift of C(21)-proton signal of ajmaline could not be detected. From the results of these spectra, it could be considered that the carbonyl group of pyrrolidone ring of PVP forms hydrogen bond with C(21)-hydroxyl group of ajmaline. When little change in infrared spectra of ajmaline by PVP is taken into consideration, this hydrogen bond seems to be extremely weak. Some indirect influence on the chromophore of ajmaline due to this weak hydrogen bond formation might give a slight shift in UV absorption spectra of ajmaline (Fig. 2).

In coprecipitate of diacetylajmaline in which the interaction with PVP could not be detected as far as spectroscopic investigation was concerned, the dissolution rate was enhanced contrary to expectation (Fig. 4). However, the enhanced ratio was very small compared with those of coprecipitates of ajmaline.

It was expected that the investigation for the binding characteristics of PVP and ajmaline or diacetylajmaline in aqueous solution might be available to discuss those in chloroform.

10) M. Muquet, J.L. Pousset, and J. Poisson, *C. R. Acad. Sci., Paris, Ser, C*, **266**, 1542 (1968).

The λ_{max} of ajmaline, diacetylajmaline, or N-methylindoline in aqueous solution remained unchanged by addition of PVP or 2-pyrrolidone, but in the spectrum of N-methylindoline the hypochromicity was observed by addition of PVP. It seemed difficult to associate directly this phenomenon of N-methylindoline with the shift to shorter wavelength in UV absorption spectra of ajmaline by addition of PVP (Fig. 2). Subsequently, the solubility and equilibrium dialysis studies for ajmaline, and the equilibrium dialysis study for diacetylajmaline were carried out. These results suggest that two or three molecules of ajmaline or diacetylajmaline may interact with one molecule of PVP.

The binding constants (K) of ajmaline and diacetylajmaline were determined from the slopes of the Langmuir plots obtained at different temperatures. Then enthalpy change (ΔH), free energy change (ΔG), and unitary entropy change (ΔSu) were calculated in the usual way and are listed in Table I. According to Molyneux, *et al.*[11] these interactions were attributed to the large unitary entropy change accompanying the disruption of the iceberg structure of water molecules around hydrocarbon groups in aqueous solution.

Table I. Thermodynamic Data for Interaction of Ajmaline and Diacetylajmaline with PVP in Water

Drug	Temp.	Binding constant (K) l/mol	Enthalpy change (ΔH) cal/mol	Free energy change (ΔG) cal/mol	Entropy change (ΔSu) cal·deg/mol	Number of binding sites
Ajmaline	0°	38.6	2300	−1980	23.7	2.8
	15°	42.4	2300	−2140	23.4	2.9
	30°	59.0	2300	−2450	23.7	2.5
Diacetate	0°	14.6	1060	−1450	17.2	2.6
	30°	17.7	1060	−1730	17.2	2.2

Results are expressed as the mean of three experiments.

From these points of view, ajmaline molecules would be bound along PVP chain by taking advantage of hydrogen bond and form solid solution in coprecipitates. Most molecules of ajmaline would be present as bound form, and unbound form would decrease with the increase in PVP content. In physical mixture, only a portion of ajmaline molecules would interact with the neighbouring PVP in aqueous solution. The number of ajmaline molecules which can interact would be limited. Concerning the dissolution characteristics of diacetylajmaline, the following consideration may be made; the diacetylajmaline molecules in solid solution are surrouded by PVP molecules, but in aqueous solution diacetylajmaline presumably tend to separate from PVP and then crystalize gradually because of the absence of hydrogen bond. The slight increase in solubility of diacetylajmaline in physical mixture would be supported by the thermodynamic data (Table I), but the small difference between the solubility of coprecipitate and that of physical mixture after 60 minutes could not be elucidated.

11) P. Molyneux and H.P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).